

CHAPTER III

EXPERIMENTAL

3.1 Materials

L-lactide was recrystallized with ethyl acetate and vacuum dried prior to use. Glycidol was distilled under reduced pressure before use. The initiators and other chemicals were used as received without further purification. Commercial-grade solvents were distilled before use.

L-lactide	: Purac Biochem
Glycidol	: Aldrich
Tin(II) 2-ethylhexanoate	: Aldrich
Boron trifluoride etherate	: Fluka
Trifluoroacetic anhydride	: Aldrich
Sodium hydrogen carbonate	: Riedel-de-Haën
Dichloromethane (commercial grade)	: Merck
Methanol (commercial grade)	: Merck
Ethyl acetate (commercial grade)	: Merck
Tetrahydrofuran (AR grade)	: Merck
Tetrahydrofuran (HPLC grade)	: Merck
Diethyl ether (AR grade)	: Merck
Hydrochloric acid	: Merck
Sodium hydroxide	: Merck
Cationic exchange resin Amberlite IRA-402	: Supleco
Anionic exchange resin Comex 50 w x 2-100	: Aldrich
Chloroform-d	: Aldrich
Deuterium oxide	: Aldrich
Nitrogen gas	: Lab Center

3.2 Instruments

3.2.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance analyses were carried out by using Varian Mercury-400 spectrometer operating at 400 MHz in deuterated chloroform (CDCl_3) or deuterated oxide (D_2O). Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) by using the residual protonated solvent signal as a reference.

3.2.2 Gel Permeation Chromatography (GPC)

Gel permeation chromatograms of PLLA and copolymer of LLA and G were obtained from Waters 600 controller chromatograph equipped with three HR (Waters) columns (HR1, HR3, and HR4) (MW resolving range = 100-500,000) at 35 °C and a refractive index detector (Waters 2414). Tetrahydrofuran was used as an eluent with the flow rate of 1.0 mL/min. The sample injection volume was 50 μL . Polystyrenes (MW = 530-43,000) were used as standards for calibration.

3.2.3 Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectroscopy (MALDI-TOF-MS)

All MS spectra of PG were acquired using BULEX MALDI-TOF mass spectrometer. Spectra were acquired in the positive-ion mode using the reflectron. The sample was dissolved in DI water and mixed with 15 mg/mL matrix solution prepared from dissolving α -hydroxycyanocinnamic acid in tetrahydrofuran. Neurotensin was used as a standard.

3.2.4 Differential Scanning Calorimetry (DSC)

Measurements were carried out on a Mettler Toledo DSC822^e. The sample was subjected to two consecutive heating scans in order to ensure an identical thermal history for the sample. All scans were measured under nitrogen.

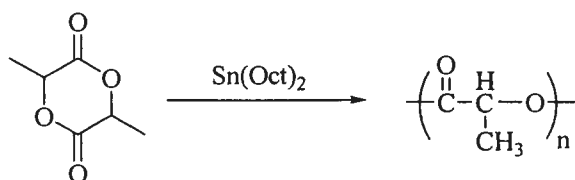
In the first scan, PG was heated at the rate of 20 °C/min from -65 to 30 °C ranges the expected T_g and then rapidly cooled to -65 °C. After the temperature was kept isothermal for 5 min, the second scan was performed at the rate of 20 °C/min to 30 °C. In case of PLLA, the sample was heated at the rate of 20 °C/min from -30 to 220 °C ranges the expected T_g and then rapidly cooled to -30 °C. The second scan was performed immediately thereafter at the rate of 20 °C/min to 220 °C. For the block copolymer, the sample was heated at the rate of 20 °C/min from -65 to 220 °C and then rapidly cooled to -65 °C. After the temperature was kept isothermal for 5 min, the second scan was performed at the rate of 20 °C/min to 220 °C. The T_g of all samples was determined in the second scan at the midpoint of the endothermic changes associated with the glass transition.

3.2.5 Contact Angle Measurements

Contact angle goniometer model Ramé-Hart 100-00 was used for the determination of water contact angles. A droplet of testing nanopure water was placed on the tested surface by bringing the surface into contact with a droplet suspended from a needle of the syringe. A silhouette image of droplet was projected on the screen and the angle was measured.

3.3 Experimental Section

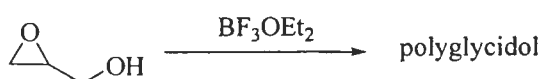
3.3.1 Polymerization of Poly(L-lactide) (PLLA)



Scheme 3.1 Polymerization of LLA.

L-lactide and $\text{Sn}(\text{Oct})_2$ (0.3 and 0.5 mol% of L-lactide) was stirred at 130 °C for 1 day. The crude product was dissolved in dichloromethane and precipitated in methanol. White solid was separated by centrifuge and dried under vacuum at 50°C.

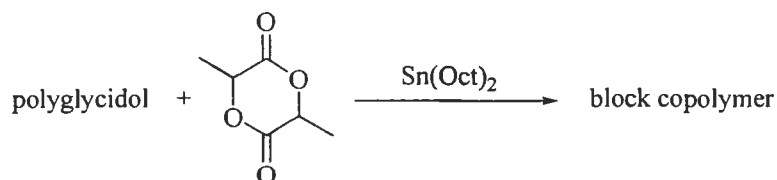
3.3.2 Polymerization of Glycidol



Scheme 3.2 Polymerization of glycidol

Sunsaneeyametha [22] investigated the effect of the addition sequence of the reagent and found that three steps of G addition result in highest molecular weight of PG. Polymerizations were carried out under nitrogen atmosphere using a glove bag. G was distilled under reduced pressure before use. $\text{BF}_3 \cdot \text{OEt}_2$ (0.08 mmol, 0.06 mol% of G) was added to small amount of G (0.03 mol) under nitrogen atmosphere. The mixture was kept in an ethylene glycol cold bath (-15 °C) for 1 h. The second addition step of G, G (0.05 mol) was slowly added to the mixture under nitrogen atmosphere. After kept at -15 °C for another 1 h, the third G (0.05 mol) was slowly added to the mixture under nitrogen atmosphere and kept at -15 °C until the reaction was complete (2 days). The resulting product was dissolved in DI water and neutralized by 10 % w/v NaHCO_3 and then passed through cationic and anionic exchange resins. After removing the DI water, transparent, colorless and highly viscous liquid was received. Polyglycidol yield was above 80%.

3.3.3 Polymerization of Poly(L-lactide-*block*-glycidol) (PLLA-*b*-PG)



Scheme 3.3 Copolymerization of LLA and PG using Sn(Oct)₂ as an initiator.

Before polymerization, trace of water was removed from PG by using toluene as azeotropic solvent. PG was dispersed in toluene which was later removed by rotary evaporation with temperature set at 50°C. The polymer was further dried under vacuum before used. The monomer molar ratios of LLA:G were listed in Table 3.1. LLA and PG were added to the round-bottom flask in a glove box purged with nitrogen gas. The flask was placed in an oil bath at 130 - 150°C until all reactants turned liquid and thoroughly mixed. Then Sn(Oct)₂ was slowly added to the mixture. The amount of Sn(Oct)₂ was 5 % by mole of the total hydroxyl group in PG. Stirring and heating were continued for 1 day. The crude product was dissolved in a small amount of dichloromethane. The solution was then centrifuged to separate undissolved initiator residue. The solution was poured into a large amount of methanol to precipitate the copolymer as light yellow solid. The removal of solvents from the remaining solution resulted in yellow solid. The products from both portions were separately purified by stirring in diethylether and dried under vacuum at 50°C. The same procedure as described above was followed using the polymerization condition listed in Table 3.1.

Table 3.1 Polymerization condition for PLLA-*b*-PG

Polymerization conditions	
Drying agent	diethyl ether, toluene, and <i>n</i> -butanol
Polymerization temperature	130, 140, and 150 °C
Polymerization time	1, 4, and 7 days
Mole ratio of G:LLA	1:2, 1:5, and 1:10
Amount of Sn(Oct) ₂	2, 5, and 10 % by mol of the hydroxyl group in PG

3.3.4 The Fractionation of PLLA-*b*-PG

The copolymer was first extracted by stirring in 20/80 EtOAc/MeOH for 30 min. The leftover solid was separated by centrifugation. Then the solids were repeatedly extracted by 40/60, 60/40, and 80/20 EtOAc/MeOH. Solids from each fractionating step were characterized by GPC and NMR spectroscopy.

3.3.5 Preparation of Polymer Films

The PLLA-*b*-PG and PLLA films were prepared by solvent-casting method. The polymer was cast from 6 wt% dichloromethane solutions by pouring into a circular glass petri dish. The solvent was allowed to evaporate for 12 h. The obtained films were further dried under vacuum at room temperature for 48 h.

3.3.6 *In vitro* Degradation

Due to low molecular weight, decent film specimens to be used in the degradation study could not be obtained. Nevertheless, the degradation behavior was studied from the fractured film pieces of either PLLA-*b*-PG or PLLA. An amount of 100 mg of each polymer was put into vial and then incubated in 10 ml of 0.01 M phosphate buffer saline (PBS) solution (pH 7.4) at 37°C. The buffer solution was replaced every week. At pre-determined periods, the phosphate buffer was removed. The incubated sample was dried in vacuum for 2 days. The sample dry weight (W_d) was compared to the initial weight (W_0). The value for weight loss was calculated by Eq. 3.1.

$$\% \text{Weight loss} = \frac{(W_0 - W_d) \times 100}{W_0} \quad \text{Eq. 3.1}$$

Molecular weight and chemical composition were also determined.